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(54) Title: PROCESS FOR PREPARING PUREE WITHOUT SYNERESIS

(57) Abstract: The present invention relates to an enzymatic method of producing purees from plant material, in high yields, which can be concentrated and then reconstituted without syneresis. Such enzymatic processing are useful for producing reconstituted pastes, purees and sauce of high quality.

Process For Preparing Puree Without Syneresis

Technical field

The present invention relates to an enzymatic process for the preparation of puree without syneresis.

Background

Any fruit or vegetable material that is finely mashed to a smooth, thick consistency is known as puree. Purees can be used as a garnish, served as a side dish or added as a thickener to sauces or soups. During fruit juice and puree manufacture enzyme preparations are often used in the steps of extraction, liquefaction, clarification and other stages. The commercial enzyme preparations contain a mixture of mainly pectinase - polygalacturonase, pectin esterase, pectin transeliminase; with minor quantities of hydrolytic enzymes such as arabinases, galactanes and xylanase.

Pectins occur in nature as constituents of higher plant cell walls. They are found in the primary cell wall and middle lamella where they are embedded in cellulose fibrils. The composition of pectin and the degree of methylation is variable among plant species and moreover depend on the age and maturity of the fruit.

Pectinases can degrade the carbohydrate polymer either by hydrolysis of the alpha 1,4-glycosidic bond (endo and exo-polygalacturonases or by transelimination reaction (pectin lyases). Pectin esterases can demethylate highly esterified pectin into polygalacturonic acid. Pectin lyases are specific for highly esterified pectins, polygalacturonases hydrolyse low esterified pectins. Consequently highly esterified

pectins can be degraded by pectin lyases or the combination of pectin esterases and polygalacturonases.

In the various stages of fruit and vegetable processing pectinases play an important role. Originally pectinases were used for treatment of soft fruit to ensure high yields of juice and pigments upon pressing and to clarify raw press juices. Polygalacturonases are used as macerating enzymes for the production of pulpy nectars, loose cell suspensions that are the result of limited pectin breakdown particularly in the middle lamella. A combination of several pectinases together with cellulolytic enzymes is needed to almost completely liquefy fruit tissue, thereby facilitating extraction. The clarification of apple juices can for example be improved by the combined activity of pectin esterases and polygalacturonases or by pectin lyases for which the highly esterified apple pectin is an ideal substrate.

Most of the pectinases present in commercial preparations are of fungal origin. *Aspergillus niger* is the most important organism for the industrial production of pectin degrading enzymes. In *A. niger* the various pectinases are not expressed constitutively. Pectin or degradation products of the pectin molecule are needed as inducing substances. The fermentation conditions for pectinase production often result in a wide spectrum of pectinases. Moreover, *A. niger* produces many isoenzymes of the various pectinases. Patents have been published describing that genes encoding polygalacturonase (EPO 0421 919, EPO 0 388 593), pectin lyases (EPO 0 278 355, EPO 0 353 188) and pectin esterases (EPO 0 388 593) have been isolated and used for the construction of overproducing transformants. These transformants allow the production of specific enzymes, needed e.g. in maceration applications and in studies on the effect of the various pectinases in processes like liquefaction and clarification.

PCT application WO 95/34223 claims a method of producing a cloud stable extract such as juices from plant material by using one or more enzymes that attack the hairy regions of pectin.

The applications of an esterase substantially free from depolymerase activity in the maceration of fruit and vegetable material is disclosed in US 5,578,335.

US 5,902,616 claims a process for preparing fresh tomato product with the help of pectinase enzyme, but without prior deactivation of the native pectin enzyme before the treatment of the pectinase. WO 96/11588 claims a process for the preparation of a tomato based product of high quality and consistency employing pectin methyl esterase enzyme.

It is advantageous to produce concentrate from a variety of plant materials, that can be reconstituted at the users end to pastes, purees and sauces. The concentrate on reconstitution is preferable to be of the desired consistency as indicated by viscosity, flow properties and lack of watery separation of the serum, which is referred as syneresis. Some of these demands can be met either through process improvements or by the use of enzymes.

Disclosure of the invention

The object of the present invention is to provide for an enzymatic process for the preparation of puree, in high yields, which on concentration followed by reconstitution shows no syneresis i.e. no separation of watery serum.

Accordingly the present invention provides an enzymatic process for the preparation of puree, characterized by-

(a) inactivation of the native enzyme of a plant material,

(b) maceration of the plant material,

(c) treating with an enzyme for 2 - 60 minutes at 25 - 50°C,

(d) inactivation of the enzyme action,

(e) optionally removing the skin and seeds,

(f) concentration to 40 to 45 Bx; and holding for subsequent processing as a concentrate,

(g) reconstitution of the concentrate with water to yield a puree with no syneresis.

The plant material is fruit or vegetable.

The fruit or vegetable is selected from apples, pears, carrots, beans, tomatoes, grapes, berries, and mangoes.

The inactivation of the native enzyme in step (a) is carried out by heating.

The maceration is carried out by grinding or milling.

The enzyme in step (c) may be a single enzyme or an enzyme mixture.

The enzyme in step (c) is dosed at 50 to 3000 ppm.

The single enzyme or the enzyme mixture is from a fungal source.

The fungus is from the *Aspergillus* sp.

The enzyme is MAPase; which is used to denote an enzyme activity that predominately attacks the side branches of the hairy regions of protopectin and pectin molecules.

The inactivation of the enzyme in step (d) in the mixture is carried out by heating.

The enzyme treated mixture to 45 Bx is carried out by heating with the application of vacuum.

The reconstitution is carried out by adding water to 9 - 25 Bx.

The enzyme mixture is obtained by a single fermentation of a non-genetically modified fungal organism.

The puree obtained is further processed to produce a sauce.

The sauce obtained is of pulpy and non-gel texture.

The present invention has the following advantages over the other reported methods:

1. No syneresis or separation of serum.
2. No loss of the desirable properties like consistency, change in viscosity, etc.
3. Increased holding capacities due to reduced volumes.
4. The puree is rich in colour and flavor.
5. The sauce produced has a pulpy and non-gel texture.
6. High efficiency of the process resulting from high yields.
7. High industrial applicability.
8. Uses an enzyme from a non GMO (genetically modified organism) source.

The invention will now be described with reference to the following examples.

Example 1 (Inactivation of the native enzyme).

Red tomato fruits are chopped, heated and pureed to inactivate the native enzyme of the tomato.

Example 2 (Inactivation of the native enzyme).

Red tomato fruits are blanched, cooled, chopped, pureed and heated to inactivate the native enzyme of the tomato.

Example 3 (Obtaining a macerated form).

The native enzyme inactivated sample obtained from Example 1 is macerated by grinding to obtain a macerated form of the tomato fruit.

Example 4 (Obtaining a macerated form).

The native enzyme inactivated sample obtained from Example 2 is macerated by milling to obtain a macerated form of the tomato fruit.

Example 5 (Production of enzyme mixture by single fermentation).

10 g of wheat bran is moistened and sterilized at 121°C at 15 psi for 30 minutes. On cooling it is inoculated with 6 ml of slant suspension of *Aspergillus niger* BICC 0425. and incubated at 30°C for 5 days. The fermented bran is extracted with 1000 ml water and used as the enzyme mixture.

Example 6 (Obtaining a concentrate).

100 g of the macerated tomato as obtained from Example 3 is treated 0.10 ml of enzyme mixture obtained from Example 5 at 50°C for 10 minutes, the enzyme mixture then inactivated by heating the mixture to 100°C. The resulting puree is

sieved to separate the skin and seeds (waste) and concentrated by heating under vacuum. A simultaneous control run is carried out without using the enzyme mixture. The following observations are made with the concentrate -

	Sample	Control
Enzyme mixture	present	absent
Waste (%)	5.87	6.27
Recovery (%)	90.90	85.40
Concentrate (Bx)	45	21

Example 7 (Reconstitution to get puree).

The concentrates obtained from Example 6 is reconstituted to 9 Bx by the addition of water. The following observations are made.

	Sample	Control
Enzyme mixture	present	absent
Viscosity (cP)	9988	9012
Synerisis	absent	absent

We claim:

1. An enzymatic process for the preparation of a puree without syneresis comprising of -
   (a) inactivation of the native enzyme of a plant material,
   (b) maceration of the plant material,
   (c) treating with pectinase enzyme for 2 - 60 minutes at 25 - 50°C,
   (d) inactivation of the enzyme action,
   (e) optionally removing the skin and seeds,
   (f) concentration to 40 to 45 Bx; and holding for subsequent processing as a concentrate,
   (g) reconstitution of the concentrate with water to yield a puree with no syneresis.
2. The method of claim 1, where the plant material is fruit or vegetable.
3. The method of claim 2, where the fruit or vegetable is selected from apples, pears, carrots, beans, tomatoes, grapes, berries, mangoes.
4. The method in claim 1 step (a), where the inactivation of the native enzyme is carried out by heating.
5. The method in claim 1, where the maceration is carried out by grinding or milling.
6. The method in claim 1 step (c), where the pectinase enzyme may be a single enzyme or an enzyme mixture.
7. The method in claim 1 step (c), where enzyme is dosed at 50 – 3000 ppm.
8. The method in claim 6, where the single enzyme or the enzyme mixture is from a fungal source.
9. The method in claim 8, where the fungus is from the *Aspergillus* sp.
10. The method in claim 1, where the enzyme is MAPase.
11. The method in claim 1 step (d), where the inactivation of the enzyme in the mixture is carried out by heating.

12. The method in claim 1, where concentration of the enzyme treated mixture to 45 Bx is carried out by heating with the application of vacuum.

13. The method in claim 1, where the reconstitution is carried out by adding water to 9 - 25 Bx.

14. The method in claim 8, where the enzyme mixture is obtained by a single fermentation of a non-genetically modified fungal organism.

15. The method in claim 1, where the puree obtained is further processed to produce a sauce.

16. The method in claim 15, the sauce obtained is of pulpy and non-gel texture.

INTERNATIONAL SEARCH REPORT

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A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A23L1/06 A23L1/212

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
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Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
WPI Data, PAJ, EPO-Internal, FSTA

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

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International Application No
PCT/IN 01/00052

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